REMARKS

Favorable reconsideration is respectfully requested in view of the foregoing amendments and the following remarks.

Applicants are submitting the present Amendment without prejudice to the subsequent prosecution of claims to some or all of the subject matter which might be disclaimed by virtue of this paper (although none is believed to be), and explicitly reserve the right to pursue some or all of such subject matter, in Divisional or Continuation Applications.

I. CLAIM STATUS AND AMENDMENTS

Claims 37-57, 67 and 68 were pending in this application when last examined and stand rejected.

Claim 37 is amended, in a non-narrowing way, to better conform to U.S. claim form. Support can be found in the disclosure, for example, at page 16, lines 11-27, page 17, line 16 to page 18, line 16, and in original claim 37.

In addition, other minor editorial revisions have been made to the claims to better conform to U.S. claim form. The revisions are non-substantive and not intended to narrow the scope of protection. Such revisions include: revising the claims to use correct U.S. punctuation and grammar throughout; correcting typographical errors; replacing the "characterized by" language with "wherein" or "in which"; and revising the language

throughout to provide proper antecedent basis for the recited terminology.

Claims 47 and 68 have been amended to change dependency to claims 42 and 37, respectively, to thus provide proper antecedent basis for the recited terminology.

Support for the amendment to claim 49 can be found in the disclosure, for example, at page 23, lines 12-16.

No new matter has been added by the above claim amendments.

Claim 67 has been cancelled without prejudice or disclaimer thereto. Applicants reserve the right to file a continuation or divisional application on any cancelled subject matter.

New claim 71 has been added. Support can be found in claims 37 and 49. No new matter has been added.

Claims 37-57, 68 and 71 are pending upon entry of this amendment.

Applicants note that the above claim amendments are intended to address matters of form only as they are not intended to affect the scope of the claims. Accordingly, if the next Office Action on the merits includes a new rejection of one or more claims, the Action must be non-final.

Applicants thank the Examiner for the careful examination of this case and respectfully request reexamination and reconsideration of the case, as amended. Below Applicants

address the rejections levied in the Office Action and explain why the rejections are not applicable to the pending claims as amended.

II. CLAIM OBJECTION

Claim 40 was objected to for containing a minor informality for the reason set forth on page 2 of the Office Action. The present amendment corrects the noted error. Thus, withdrawal of the objection is solicited.

III. INDEFINITENESS REJECTION

Claims 37-57, 67 and 68 were rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for the reasons set forth on pages 2-4 of the Office Action. This rejection is respectfully traversed as applied to the amended claims.

Claim 37 has been amended to remove the term "about" from the phrase "at least about double on a logarithmic scale" at the end of the claim. This amendment renders moot the concern raised at the bottom of page 2 of the Action.

Claim 49 has been amended to address the concern regarding "microparticles" at the top of page 3 of the Action. Specifically, amended claim 49 specifies that the method further comprises separating micro particles from the sample based on their scattering and/or fluorescence properties. These micro particles can also be present in the sample. Support for such

can be found in the disclosure, for example, at page 23, lines 12-16.

Claim 55 is amended to specify that the sample is from a mammal, thus overcoming the rejection in the middle of page 3 of the Action.

Claim 67 has been canceled without prejudice or disclaimer, thus rendering moot the concern at the bottom of page 3 of the Action.

Claims 37, 41 and 49 have been amended to remove the lack of antecedent basis concerns set forth on pages 3-4 of the Action.

The amended claims are thus clear, definite and have full antecedent basis.

This rejection is believed to be overcome, and withdrawal thereof if respectfully requested.

IV. PRIOR ART REJECTIONS

Claims 37-48, 50-53, 55-57 and 67-88 were rejected under 35 U.S.C. § 102(b) as anticipated by SCHUT et al. (WO 99/10533) for the reasons on pages 4-6 of the Office Action.

Claims 37-53, 55-57 and 67-88 were rejected under 35 U.S.C. § 103(a) as being obvious over SCHUT et al. in view of MATSUMOTO et al. (U.S. 5,888,823) for the reasons on pages 6-7 of the Action.

Claim 54 was rejected under 35 U.S.C. § 103(a) as being obvious over SCHUT et al. in view of MATSUMOTO et al. and WALLNER et al. (Cytometry 1993) for the reasons on pages 8-10 of the Action.

These rejections are respectfully traversed and will be discussed together below.

It is well established that to anticipate a claim, a cited prior art reference must disclose or suggest each and every element of the claimed invention. *See*, M.P.E.P. (Eighth Ed., Rev. 6 (September 2007) at § 2131.

It is well also established that to support a *prima facie* case of obviousness, the Office must provide a rationale showing that all the claimed elements were known in the prior art and one skilled in the art could have combined the elements as claimed by known methods with no change in their respective functions to yield predictable results. *See, KSR International Co. v. Teleflex Inc.*, 550 U.S. ___, ___, 82 U.S.P.Q.2d 1385, 1395 (2007); and M.P.E.P. (Eighth Ed., Rev. 6 (September 2007) at § 2143.02.

In the instant case, amended claim 37 recites:

"A method for identifying at least one micro-organism and/or micro-organism species in a sample, and for measuring the portion of the identified said at least one micro-organism and/or micro-organism species from the sample, said method comprising:

a) binding a first fluorescent agent that absorbs light in a first wavelength area to a structure individualizing at least one micro-organism species or group in said sample and enabling identification thereof,

b) binding a second fluorescent agent that absorbs light in a second wavelength area to a structure characteristic of all micro organisms,

c) subjecting the sample to flow,

d) exciting the first fluorescent agent in the flow with a monochromatic light disposed in the first wavelength area,

e) exciting the second fluorescent agent in the flow with a monochromatic light disposed in the second wavelength area, and

f) identifying a target micro-organism by analyzing fluorescence of the first and second fluorescent agents bound to particles of the sample,

wherein the first and second fluorescent agents and the wavelength areas of the monochromatic light are chosen in such a manner that the difference in intensities of the mean fluorescences of the first and second fluorescent agents is at least double on a logarithmic scale."

Applicants respectfully submit that SCHUT et al., MATSUMOTO et al. or WALLNER et al., taken alone or in combination, fail to disclose or suggest the method of claim 37 in which fluorescent agents and wave-lengths of the monochromatic

light are chosen in such a manner that the difference in intensities of the mean fluorescences of the first and second fluorescent agents is at least double on a logarithmic scale.

SCHUT et al. disclose a method for use in *in situ* staining of micro-organisms comprising: a) mixing a material containing at least one (fixed or non-fixed) micro-organism with a composition which can (partly) degrade a cell wall or cell membrane of micro-organism thereby allowing for penetration through said wall and/or membrane of a (labeled) probe into said micro-organism, b) optionally fixing said micro-organism to further retain its corpuscular character, c) reacting said probe with an antigen or nucleic acid molecule present in said micro-organism, and d) detecting the presence of said probe in said micro-organism. The composition comprises at least one detergent and at least one enzyme or one enzyme.

SCHUT et al. claim that the use of various enzymes is the only way to have adequate separation between a probe negative and a probe positive population.

In the Office Action, the Examiner has referred to a method describing a conventional FISH method disclosed in, for example page 36, line 10 to page 37 line 15, page 53, and figure 5, and page 52, and figure 4, which does not use enzymes. However analysis of this kind of data (in Fig. 5, lower line) is synonymous to guessing the result. It is not predictive of the invention of claim 37. This is evident from the fact that

one can clearly see that there is absolutely no separation between the marked NEG-population and the POS-population. Actually both claimed populations seem to belong to the same single population NEG+POS. The DNA stains that are picked up by this method are quite bad. It can clearly be seen that there is no correlation with the DNA stain and 16S rRNA probe stain intensities (Fig. 5).

Several problems of this "conventional" FISH method are already listed in pages 5 to 6 of SCHUT et al. and include the following (1)-(3):

(1) A major problem to overcome when wanting to apply flow cytometry is maintaining sufficient integrity of the individual cells which need to be tested while at the same time making the content of cells accessible to the necessary probes. Fragmented or disintegrated cells cannot be detected as an individual cell with flow cytometry. It is essential that the corpuscular character of the cell is maintained. See page 5, 14-21 of SCHUT et al.

(2) There is a need for staining techniques allowing the detection of specific traits of individual whole cells in solution, especially cells surrounded by a cell wall or cell envelope that have in general not been accessible to such techniques. This makes it impossible for labeled probes to penetrate the cell and react with its specific antigen or nucleic acid. See page 5, lines 21-32 of SCHUT et al.

(3) Labeled probes are needed that can penetrate the cell envelope and cell membrane and can react specifically *in situ* with antigens or nucleic acids. Methods are in general only applicable for a limited number of, or even only one, species of micro-organisms. Methods allow only little or no penetration of probes into the cell. See page 5, line 34 to page 6, line 13 of SCHUT et al.

In addition, some of these problems of conventional FISH are also mentioned in the present application. See, for instance, the description in paragraphs [0011] and [00121) of the instant application. These methods, known at present, are not suitable for routine use and they cannot be used to calculate the microorganism concentrations of mixed microorganism samples. Further these methods do not calculate the concentrations of the bacteria per unit of volume, but only the proportions of the bacterial species.

SCHUT et al. do not either disclose how to use their enzyme treatments to any real complex microbial sample like a fecal sample. They only show that their enzyme treatment can be used for pure cultured LD-BOS cheese starter culture (Example 7). SCHUT et al. does not disclose or suggest how to analyze a complex microbial sample as achieved by the present invention of amended claim 37.

Further, SCHUT et al. do not encourage at all following the path the Applicant has gone done. In this regard, SCHUT et

al. disclose the use of various enzymes is the only way to achieve adequate separation between a probe negative and probe positive population. The method of the present invention as in claim 37 does not rely on enzymes.

Accordingly, it is believed that SCHUT et al. does not disclose or suggest each and every element of amended independent claim 37. Also, it would not have been predictable to alter the teachings in SCHUT et al. to arrive the method of claim 37 as evident from the problems associated with the method of SCHUT et al. Thus, SCHUT et al. cannot anticipate or render obvious claim 37.

MATSUMOTO et al. fail to remedy the above-discussed deficiencies of SCHUT et al.

MATSUMOTO et al. disclose a standard fluid to be used in the quality control and calibration of a flow cytometer. The non-biological standard fluid is free from disadvantages encountering materials with biological origins.

However, MATSUMOTO et al. have nothing in common with the method of claim 37 of the present application. In this regard, the method of claim 37 does not use or need a calibration fluid. Also, the micro-particles in the method of claim 37 are several times bigger than bacteria and smaller than eukaryotic cells. They also do not show the same fluorescence intensity and scatter light intensity as those of the cells to be assayed as in MATSUMOTO et al. which utilize their standard fluid.

As such, Applicants respectfully submit that it would not have been obvious to solve the above-discussed problems of the method of SCHUT et al. by combining it with the teachings of MATSUMOTO et al. Also, there is no motivation/reason to combine the method of SCHUT et al. with the calibration fluid of MATSUMOTO et al.

Thus, the combination of SCHUT et al. and MATSUMOTO et al. does disclose, suggest or otherwise render obvious the method of independent claim 37.

WALLNER et al. fail to remedy the above-discussed deficiencies of SCHUT et al. and MATSUMOTO et al.

WALLNER et al. disclose a method similar to that which has been cited in the description in paragraph [0012]. The method of WALLNER et al. requires the use of very strong and expensive water cooled lasers having the power of hundreds of milliwatts. The intensity of the fluorochromes used in the method of WALLNER et al. remains weak. As such, in the method of WALLNER et al., the target populations cannot be satisfactorily distinguished from each other in one analysis.

WALLNER et al. use an excitation energy wavelength of 488 nm and amount of 500 mW to excite the fluorochrome (FLUOS). They also use an excitation energy wavelength of 514 nm and amount of 500 mW to excite the fluorochrome (TRITC) bound to micro-organisms in a sample. When choosing these wavelengths WALLNER et al. have not taken into account, and did not

comprehend the influence, that when the wavelength of light decreases the energy per quantum increases and that the amount of scattering of small particles is inversely proportional to the fourth involution of the used wavelength and almost proportional to the amount and power of the laser-lines. Thus, in contrast to the method of claim 37 of the present application, in the method of WALLNER et al., due to the unfavorable wavelength ranges, a lot of energy is lost to form auto-fluorescence of auto-fluorescent components of micro-organisms and other particles in the sample, instead of exciting said fluorochromes.

Further, the wavelengths of excitation energy that WALLNER et al. employ cause a lot of auto-fluorescence in the wavelength range of 515-545 nm, i.e., at the same wavelength range at which they try to identify light emitted by fluorochromes of probes bound to the target micro-organisms. This unwanted auto-fluorescence clearly causes problems when analyzing samples and when trying to identify a specific target micro-organism. In contrast to the method of claim 37 of the instant application, WALLNER et al. did not take this fact into account in their method.

Another factor that WALLNER et al. did not take into account, is that the emission spectras of the chosen fluorochromes (FLUOS and TRITC) disturbingly overlap in the red wavelength area. This overlap will cause a significant increase in background fluorescence and harm the analysis.

Further, WALLNER et al. use only artificial samples, e.g., a mixture of rapidly growing *E. col.* and *S. carlsbergensis* cells. WALLNER et al. state that two cell types can easily be distinguished by another independent parameter, such as forward angle light scatter (page 138, Results and discussion, first paragraph). However, this statement do not hold for complex microbial samples, due to the fact that several different bacterial species can have similar scatter properties in certain conditions and even certain non-microbial particles and fibers can have similar scatter properties. The use of forward light angle scatter to distinguish different bacteria from each other becomes even more unreliable when taking into account the fact that even material with no biological origin may have bacterial scatter properties as discussed in MATSUMOTO et al. (page 6, column 2, lines 1-10).

Based on the above, Applicants respectfully submit that the combination of SCHUT et al., MATSUMOTO et al. and WALLNER et al. is not predictive of the method of claim 37.

In addition, WALLNER et al. do not show or describe using the method therein to analyze anything from complex microbial samples, e.g. fecal samples. In this regard, it is known that rapidly growing cultured cells contain more ribosomes than cells exposed to loads of competition in a complex ecosystem, such as in intestines and feces (competitive exclusion). In fact, WALLNER et al. state that target cells with

weak fluorescence signal resulting from low cellular ribosome contents or restricted cell permeability may be misinterpreted as nontarget cells (page 138, Results and discussion, second pragraph). This means that WALLNER et al. cannot reliably detect cells which have a weak fluorescence signal or contain less ribosomes, e.g., fecal sample (look also at page 141. Growth rate and ribosome content, especially last three rows).

Similar to SCHUT et al., WALLNER et al. also imply that one should use enzymes when probes show limited accessibility. See page 138, Results and discussion, second paragraph.

Even by using an artificial sample (not to mention complex microbial sample), one can easily see that there is, in practice, no separation between populations in gates #1 and #3 or between populations in gates #2 and #4 (page 142, Fig. 5. B, WALLNER et al.). In fact, WALLNER et al. state that this is due to several of problems discussed in the text.

In the method of WALLNER et al., scattering produced by the first laser (488 nm argon ion laser) and the fluorescence of the fluorochromes of the probes bound to the excited target micro-organisms are measured at the same time.

By contrast, in the method of claim 37 of the present application, the first laser (635 nm diode laser) is used only to excite the fluorescent agents of the probes bound to the target micro-organisms and scattering produced by the laser is not measured. In this way, optimizing the scattering parameters and

the fluorescence of the fluorescent agents of the probes bound to the target micro-organisms at the same time is avoided. Said parameters can be optimized separately, and as such, they have no influence on each other. This is not possible, nor is it even disclosed in the method of WALLNER et al.

Applicants further emphasize that both the "conventional" FISH method and the method of SCHUT et al., MATSUMOTO et al. and WALLNER et al. are unable to solve the problems discussed below (which the method of claim 37 does solve):

(1) How to analyze the accurate concentration, i.e., absolute count of total/all micro-organisms (ALL) from a complex microbial sample?

(2) How to analyze the accurate concentration, i.e., absolute count of the probe defined target micro-organisms (TARGET) from a complex microbial sample?

(3) How to analyze the accurate concentration, i.e., absolute count of other microorganisms (X) directly from a complex microbial sample without solving the equation ALL - TARGET = X?

(4) How to analyze the accurate concentrations, i.e., absolute counts of background particles from a complex microbial sample?

(5) How to analyze the accurate concentration, i.e., absolute count of inaccurately probe defined "false target micro

organisms (probe stained but not DNA stained particles (See present application Fig. 5; particles below target microorganisms (30) and to the right of background (29))?

(6) How to analyze accurately all these parameters from a complex microbial sample in just a single analysis and single plot and to do so without the use of enzymes.

Applicants respectfully submit that the method of claim 37 solves these problems. In the method of the present application, the problems have been solved by using a flow cytometer and by using two simultaneous threshold parameters and choosing fluorescent agents and wavelengths of the monochromatic light in such a manner that the difference in intensities of the mean fluorescences of the fluorescent agents is at least double on a logarithmic scale.

The combined teachings of SCHUT et al., MATSUMOTO et al. and WALLNER et al. in no way disclose or suggest the above discussed approach of the method of independent claim 37, involving an analytical approach for the selection of the wavelengths or the fluorescent agents.

In fact, SCHUT et al. do not encourage at all following the path the Applicant has followed. Again, SCHUT et al. disclose that the use of various enzymes is the only way to adequately reach microbial 16S rRNA molecules and have adequate separation between a probe negative and a probe positive population. In this sense, SCHUT et al. teaches away from the

method of claim 37. WALLNER et al. also suggest the use of enzymes (and certain signal amplification procedures).

In this sense, SCHUT et al. and WALLNER et al. teach away from the method of claim 37. It is well established that prior art references cannot be combined where a reference teaches away from their combination. *See*, M.P.E.P. (Eighth Ed., Rev. 6 (September 2007) at § 2145, X, D, 2. Again, the method of claim 37 does not require the use of enzymes.

The method of claim 37 enables the skilled artisan to solve the problems described above without having to employ any slow, complicating and expensive enzyme interventions/treatments and without slow and complicating post-hybridization washings that SCHUT et al. and WALLNER et al. (page 139, Nonspecific staining) are suggesting.

Until Applicants' method of claim 37, it had been impossible to acquire reliable results from this type of faint staining and from low to none separation of the negative and positive populations. For the reasons discussed above, the skilled artisan could not have used conventional data analysis tools, because the results would become distorted.

In fact, the method of claim 37 is the only commercially available method which uses flow cytometry to analyze samples, containing numerous and various micro-organisms.

The Applicants have surprisingly come up with the idea that 1) target micro-organisms, 2) other micro-organisms and

3) other particles and background/noise of a sample can be reliably separated from each other by using a flow cytometer by choosing the fluorescent agents and the wavelengths of the monochromatic light so that the difference in intensities of the mean fluorescences of the fluorescent agents is at least double on a logarithmic scale. SCHUT et al., MATSUMUTO et al. and WALLNER et al., taken alone or in combination, do not, in any way, disclose or suggest such a combination.

The Applicants surprisingly found that the said difference can, in accordance with the method of claim 37, be achieved by choosing the correct combination of the excitation energy wavelength range, fluorescent agents and wavelengths at which signals from micro organisms can be observed.

Thus, the combination of SCHUT et al. and MATSUMOTO et al. does disclose, suggest or otherwise render obvious the method of independent claim 37 and new independent claim 71. Also, in view of the above-discussed differences between the methods of the cited references and the method of claim 37, it is clear that it is clear that the method of claim 37 is novel and patentable over the cited references.

The remaining claims all depend on claim 37. Thus, they too are novel and patentable over the combined references for the same reasons due to their dependency on claim 37.

Again, the benefits achieved with the method of claim 37 make it possible for the first time possible to use

flow cytometry to analyse samples, containing numerous kinds of microorganisms, in one analysis and to demonstrate target micro-organisms, other micro-organisms and other particles of a sample in one graph.

For the above-reasons, Applicants respectfully submit that the above-noted prior art rejections are untenable and should be withdrawn.

V. CONCLUSION

In view of the foregoing amendments and remarks, it is respectfully submitted that the present application is in condition for allowance and early notice to that effect is hereby requested.

If the Examiner has any comments or proposals for expediting prosecution, please contact the undersigned attorney at the telephone number below.

The Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 25-0120 for any additional fees required under 37 C.F.R. § 1.16 or under 37 C.F.R. § 1.17.

Respectfully submitted,

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